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In Search of the Chimera: Molecular Imaging in the Atom-Probe

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The Atom-Probe Field Ion Microscope is the ultimate microanalytical tool because a single atom can be chosen from its neighbors at the discretion of the observer and identified in a time-of-flight mass spectrometer [1]. A simplification of the apparatus called the *10-cm Atom-Probe* (Fig. 1) extended the analysis capability of the original instrument [2]. With the introduction of the *Imaging Atom-Probe* a preselected species could be visualized in atomic resolution and mapped in three-dimensions with a lateral and depth resolution exceeding 0.5 nm under ideal conditions [3]. Although Atom-Probe analysis has been confined to surface studies and problems in the materials sciences the lure of imaging and analyzing individual molecules has been strong. See a review of attempts to image individual molecules in the Field-Emission Electron Microscope and the Field-Ion Microscope for details [4].

Sample Preparation

If a molecule, such as copper phthalocyanine, can be sublimed onto a substrate in high vacuum sample preparation presents no difficulty. Unfortunately, most molecules of interest (DNA, proteins or virus particles) must be deposited onto a substrate from aqueous solution and then transferred into high vacuum for imaging. Early attempts to image these molecules were hampered by an inability to *independently* verify the success of the deposition process. This obstacle was overcome by using the Transmission Electron Microscope (TEM) to verify protein deposition on field-emitter tips (Fig. 2) [5]. A refinement of the deposition protocol minimized the drying artifacts associated with surface tension forces acting on the sample as it is moved through an aqueous interface and dried in air [6]. For a molecule of characteristic dimension, d, in meters surface tension results in a pressure, $Pg \approx 0.146/d\ N/m^2$ that can redistribute molecules on the surface and distort or destroy their tertiary structure [7].

Imaging Constraints

The electric field strength generated at the substrate is the greatest obstacle to successful imaging of molecules in the Atom-Probe. The magnitude of the field, E, that can redistribute molecules on the surface and distort or destroy their tertiary structure can be estimated by assuming the outward-directed electrostatic pressure is equivalent to Pg. Then $E \approx (2Pg/\varepsilon_0)^{1/2} = (0.292/\varepsilon_0 d)^{1/2} \approx 18$ MV/cm which is at least an order of magnitude below the field strength required for imaging in hydrogen or helium.

Field Ion Tomography

Stable and reproducible images of a ferritin monolayer on a tungsten surface were obtained in the Imaging Atom-Probe by embedding ferritin within a layer of vitreous benzene ice condensed onto an 80 K surface using gas phase benzene as a *blanket gas* [4]. As the field is increased, benzene is desorbed from the surface as cluster ions $(C_6H_6)_n^+$, n=1,2) which expose the contour of ferritin molecules as a function of depth within the benzene layer (Fig. 3). This process, called *Field-Ion Tomography* can be used to reconstruct the three-dimensional morphology of a ferritin monolayer (Fig. 4) [8]. Image resolution is limited by the size of the benzene cluster ions to ≈ 2 nm and the field strength for benzene desorption (≈4 MV/cm) is well below the field required to distort, destroy, or desorb the ferritin monolayer from the surface [8]. Cryofixation in vitreous water ice and cryotransfer into the Atom-Probe has been demonstrated and could improve the resolution of the imaging process [9].

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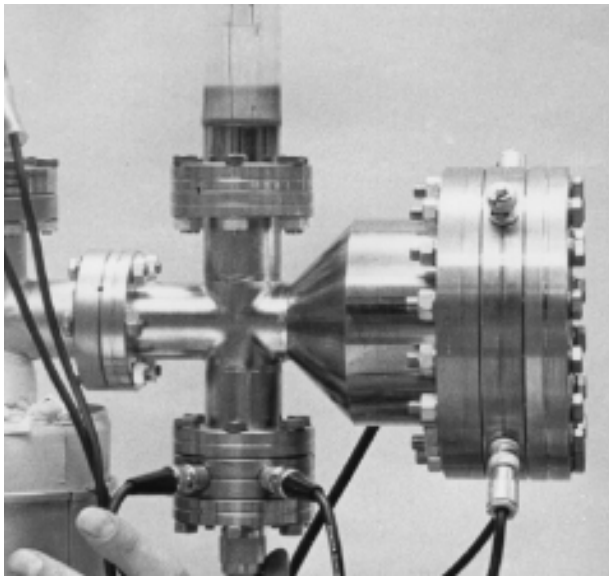

Fig. 1. The 10-cm Atom-Probe.

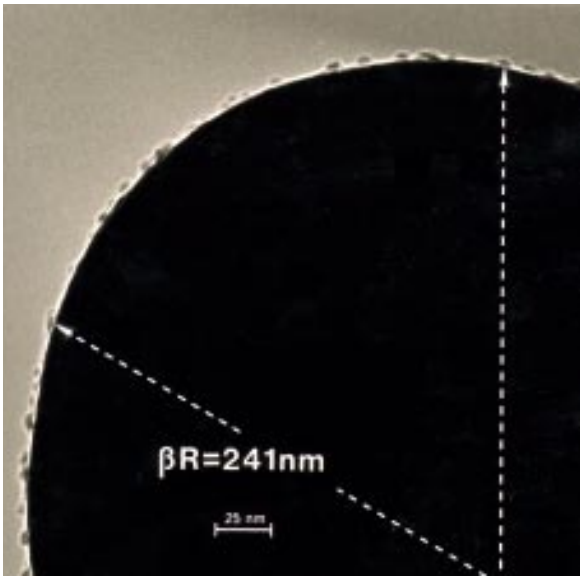

Fig. 2. A Ferritin Monolayer on Tungsten (β≈1.5).

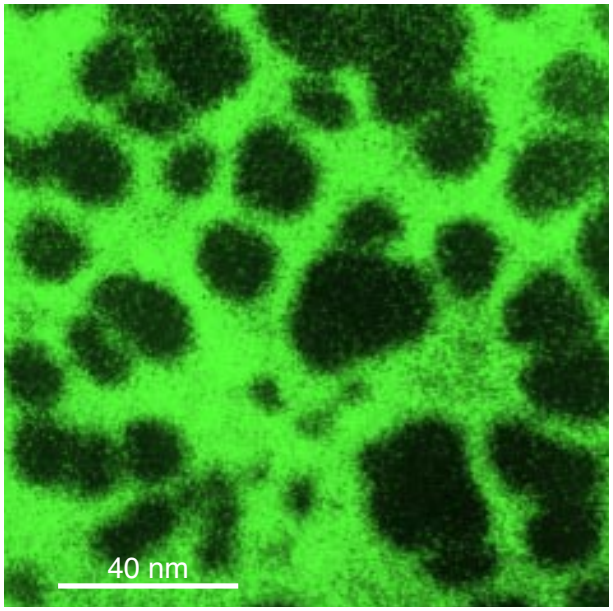

Fig.3. Field-Ion Tomography of Ferritin.

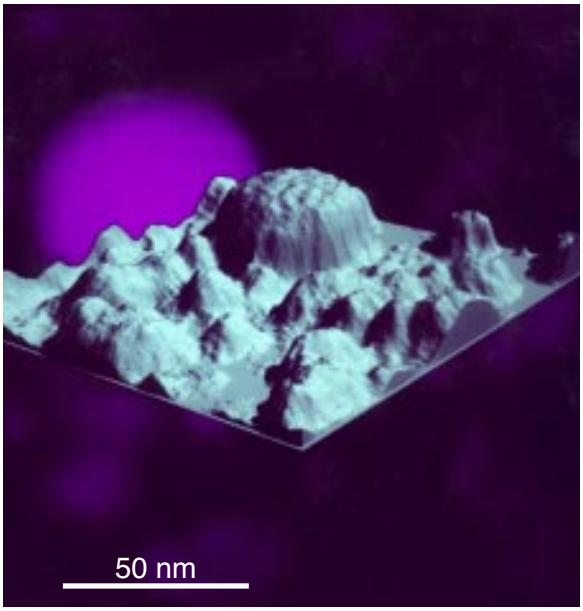

Fig. 4. A Tomgraphic Reconstruction of Ferritin.

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